

## An Ecological and Microbiological Study of Urea Fertilization and Thinning in a Black Spruce Stand

*M. R. Roberge, G. F. Weetman, and R. Knowles*

LARGE AREAS OF BOREAL FOREST in Canada exhibit humus accumulation. In addition to presenting problems of slow growth, difficult regeneration, and in some instances site degradation, such humus accumulations usually causes low rates of nutrient release to plants, despite large reserves of unavailable nutrients such as N, P, and K.

The possibility of applying the results of work done elsewhere, particularly in Europe, to solve Canadian land-management problems associated with humus accumulation, led one of the authors in 1958 to extensively survey the literature of mor humus in relation to Boreal forest conditions (36, 37, 38). Ecologically, mor humus accumulation in Boreal forests shows all or most of the following: a slow tree—humus nutrient cycling; large reserves of unavailable nutrients, especially N; high moisture content; low and uniform temperatures during the growing season; poor aeration in the humus; a permafrost layer close to the surface; low pH; high C/N ratio; high exchange capacity; low base saturation; accumulation of tree roots in the humus; an invasion by mosses that rely on air and tree drip for nutrients; the presence of few or no nitrifying bacteria; active fungal competition for nutrients and large formation of fungal-cell substances; production of organic acids, auxins, and chelating agents by fungi; presence of partially decomposed fungal-cell walls; and a lack of humification.

The purpose of this study was to examine means of increasing tree and regeneration growth and of avoiding, or at least decreasing,

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*M. R. Roberge is Research Scientist, Department of Forestry and Rural Development of Canada, Sillery, Quebec, Canada; G. F. Weetman is Research Forester, Pulp and Paper Research Institute of Canada, Pointe Claire, Quebec, Canada, and R. Knowles is Associate Professor of Microbiology, Macdonald College of McGill University, Ste-Anne de Bellevue, Quebec, Canada.*

the accumulation of humus on the mineral soil of a Boreal black spruce (*Picea mariana* Mill.) stand. The effects of urea fertilization and thinning were studied. For experimental purposes, the most useful assessment of effects of urea and thinning treatments seemed to be ways in which: 1) rate of tree growth was modified over a five-year period; 2) attempts to mobilize N reserves after clear-cutting affected the growth of planted black spruce; 3) physical environment of the humus horizon was changed; and 4) uptake and cycling of N in the stand were changed in relation to field, greenhouse, and laboratory studies of gross and net N mineralization rates in the humus.

This report summarizes the ecological and microbiological studies made in the field, greenhouse, and laboratory by the authors and their co-workers.

### Materials and Methods

A four-hectare black spruce stand 90 kilometers north of Baie-Comeau, Quebec, was treated in 1961 (39). This stand is on limits of the Quebec North Shore Paper Company, latitude  $49^{\circ}44' N$  and longitude  $68^{\circ}09' W$ , in the Laurentide-Onatchiway Section B.1a of the Boreal forest region (32). Total annual precipitation here is 90 cm, of which 40 cm falls during the growing season. The mean temperature of the 150-day growing season is  $12^{\circ}C$ . There are many unsolved problems regarding the reproduction, thinning, and fertilization of upland black spruce stands, the major cover type in this region.



Figure 1. View of the 65-year-old upland black spruce stand.

The stand lies on a well-drained, 10 percent slope with a northerly aspect. The soil is about 50 cm deep (20), and belongs to the humic podsol subgroup (21). Raw humus accumulates to a depth of about 20 cm. Parent material is composed of coarse, sandy-loam, till deposits (Figures 1 and 2).

The site is a Calliergon forest type of the *Hypno-Piceetum* association (19). It is classified as quality 2 of the northeastern Boreal forest region, with black spruce reaching 12.2 m at 50 years or 14.0 m at 100 years.



Figure 2. Podzol soil profile with a thick mor humus horizon capped with feather mosses under black spruce.

The stand was established after a severe fire in 1896. In 1961, age at stump height of the trees ranged from 45 to 65 years, indicating a 10- to 20-year regeneration period. There were 7,100 trees/ha with a mean dbh of 10.7 cm, a mean height of 13.3 m, a basal area of 42 m<sup>2</sup>/ha, and a total volume of 220 m<sup>3</sup>/ha. Growth in diameter and height was 0.93 mm and 7.0 cm, respectively. The canopy intercepted 90 percent of total light intensity and 40 percent of total rainfall (45).

A factorial experiment (Figure 3), designed to compare interactions between two or more influencing factors, was set up mainly to determine the effects of various combinations of urea applications and thinning treatments on black spruce growth and humus decomposition (39). Two thinning treatments were adopted: removal of 25 percent and 50 percent of the volume of the stand from the smaller diameter classes. These thinning treatments were carried out on 0.4-ha areas. These were subdivided into 0.13-ha plots which were treated with 0, 110, and 450 kg urea-N/ha, respectively. In addition to the two thinning treatments, a 1.2-ha plot was clear-cut and planted. All cutting, planting, and fertilizing experiments were begun in late summer, 1961.

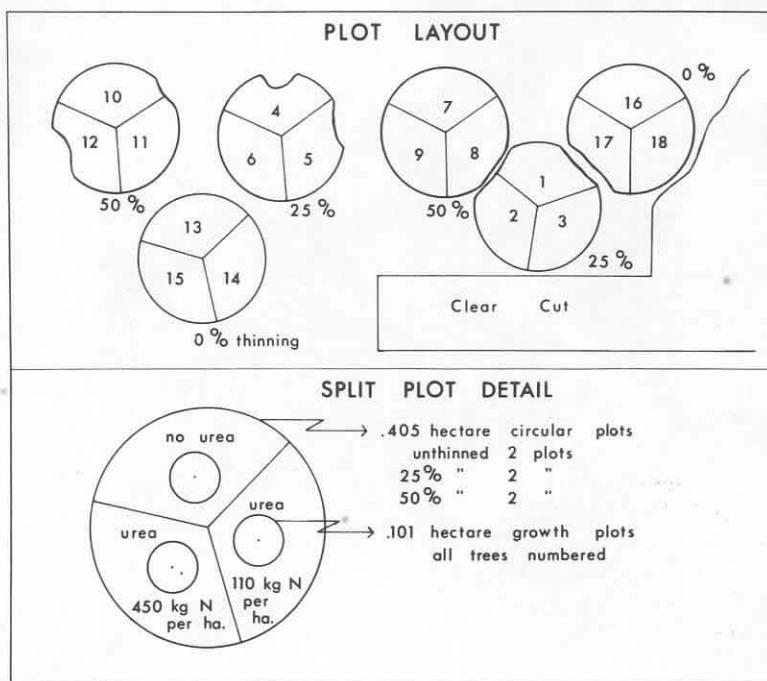


Figure 3. Plot layout in the black spruce stand.

From 1961 to 1966, a series of detailed ecological and microbiological studies were made. Tree growth rate was measured both by periodic diameter tallies and by increment core analyses (46). Tree components were weighed with a strain gauge apparatus (40). Litterfall was collected in Fourdrinier wire screens (40). Integrated light intensity was measured with ozalid paper meters (45). Rainfall distribution was measured in plastic buckets (43). Black spruce seedling growth was studied in intact cores of humus and soil placed in a constant temperature bath in the greenhouse (44). Needle decomposition rates were studied in nylon and fiberglass bags on the forest floor (43). Humus temperatures were measured with battery driven recorders attached to mercury in steel thermometers (34). Nitrogen loss by leaching was determined with tension lysimeters (47). Total N was determined by Kjeldahl analysis and  $\text{NH}_4\text{-N}$  after extraction with acidified  $\text{K}_2\text{SO}_4$  (26). Hydrolysis of urea added to 5-g quantities of humus was studied by following the liberation of  $\text{NH}_3\text{-N}$  at 20° C in the laboratory (26, 29, 30, 31). Bacteria and fungi were enumerated by the dilution-plate technique (28), and fungi were also enumerated by the soil-washing technique (22).

## Results of the Studies

### Effects of Urea Fertilization in the Unthinned Plots of the Stand

Growth and foliar nutrient concentrations of black spruce trees were measured in unthinned plots of the stand fertilized with 110 or 450 kg urea-N/ha. Moreover, microbiological analysis of the humus was carried out following fertilization with 450 kg urea-N/ha.

#### Stand

Black spruce trees showed a growth response to the N fertilization (Figures 4 and 5 and Table 1). The diameter growth increase was detectable at the end of the first growing season and was still obvious at the end of the fifth growing season after urea application (Figure 5).

Sampling of the foliage each October indicated an immediate increase in the N concentrations after the application of urea, and from then on a steady decline occurred (Figure 6). By 1966, the N concentrations in current needles of the fertilized trees were almost as low as those of the unfertilized trees, but a direct relationship was still found between the amount of N added and the N concentration found in the needles (Table 1).

In 1966, the foliar P, K, Mg, and Ca concentrations in current-year needles of trees fertilized with 450 kg urea-N/ha were equal to

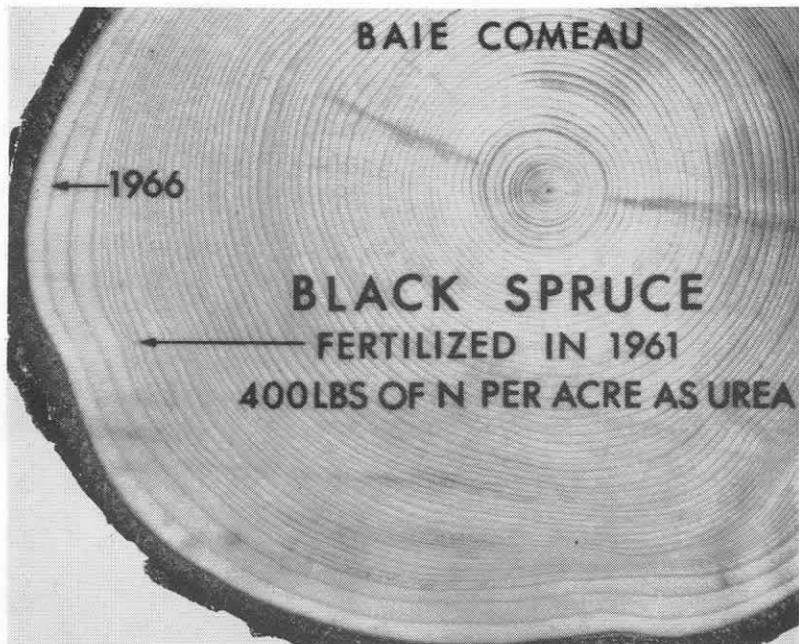


Figure 4. Diameter growth on black spruce tree fertilized with urea (400 pounds per acre = 450 kg/ha).

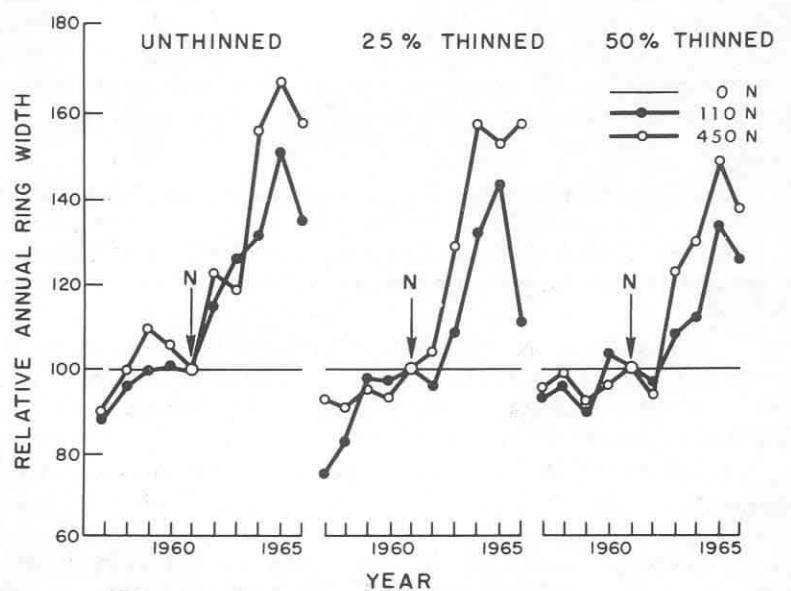


Figure 5. Width of annual rings and relative values for black spruce (base year 1961 = 100).

or slightly lower than those of unfertilized trees (Table 1). The proportional concentrations (i.e., taking the N concentration as equal to 100) of P, K, Mg, and Ca all were lower, however, with a rise in N concentration due to the 450 kg urea-N/ha application. But only K dropped slightly below the proportional level considered to be optimum (15). Other than N, there were no visual symptoms of nutrient deficiencies during the experiment.

Table 1. EFFECTS OF UREA FERTILIZATION ON TREE GROWTH AND NUTRIENT CONCENTRATIONS AND PROPORTIONS IN CURRENT NEEDLES

Urea-N added in 1961 (kg/ha)	Diameter growth 1951-66 (mm)	Nutrient concentrations (% dry wt. basis) and proportions* in current needles in 1966					
		N	P	K	Mg	Ca	
0	4.39	Conc.	0.92	0.21	0.49	0.14	0.39
		Prop.	100	23	53	15	42
110	5.99	Conc.	0.93	0.19	0.40	0.14	0.43
		Prop.	100	20	43	15	46
450	6.36	Conc.	1.12	0.18	0.45	0.15	0.29
		Prop.	100	16	40	13	26
* Optimum proportions (15)		100	8-15	50-100	5-10	5-10	

### Regeneration

In 1966, there was no black spruce regeneration even though large numbers of black spruce germinants appeared one year after application of 450 kg urea-N/ha. Nearly all of these germinants died during the following year.

### Mosses

The ground vegetation consisted mainly of feather mosses (45). Three major species were encountered: *Pleurozium schreberi*, *Hypnum crista-castrensis*, and *Dicranum rugosum*. In the plots which had received 450 kg urea-N/ha, the moss flora appeared brown and many of the moss plants died. By the second year after urea application, patches of mosses were green again. By 1966, almost all of the mosses had recovered.

### Humus horizon

In 1966, the total N concentration was higher in layers of the treated humus than in layers of the untreated humus (Table 2). As a consequence of this increase in total N concentration, C/N ratios were appreciably lower in the humus layers of the treated plots than in those of the untreated plots. It is thus likely that more N was, is, and

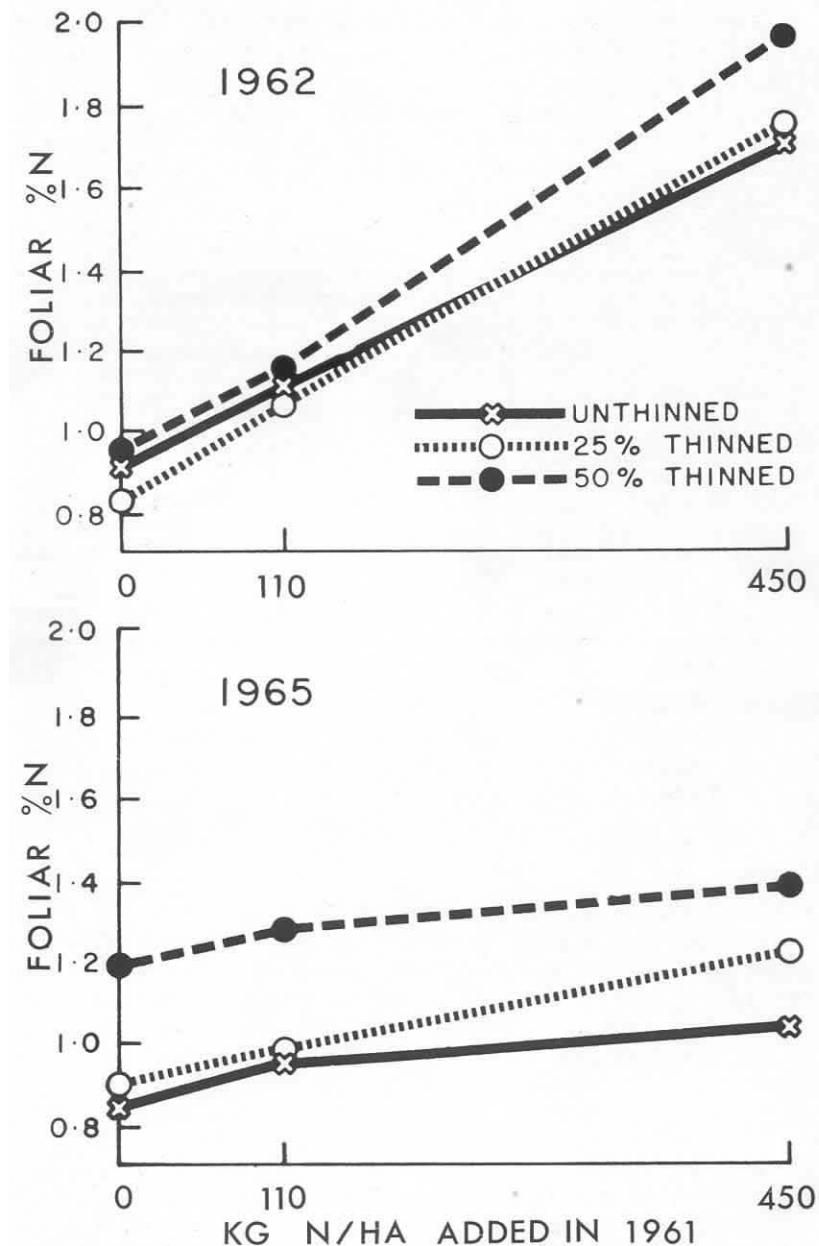


Figure 6. Relationship between foliar N level and the amount of N added as urea.

will become available for plants in the humus of the treated plots than in the untreated plots. ~~The increase in total N increased~~

In 1966, the pH in the humus layers of the 450 kg urea-N/ha fertilized plots was higher than in the humus layers of the unfertilized plots (Table 2). Two years after urea application, a maximum of pH 6.0 was noted in the L layer (24). The relative degree of availability of many essential elements increases with pH over the range encountered in this study, and for most conifers a pH value of 5 to 6 is considered satisfactory. The results suggest, therefore, that the pH increases following urea fertilization of this humus with 450 kg urea-N/ha were not excessive for tree growth.

From 1963 to 1966, amounts of urea,  $\text{NO}_2\text{-N}$ , and  $\text{NO}_3\text{-N}$  were negligible in the humus layers of the 450 kg urea-N/ha fertilized plots and in the humus layers of the unfertilized plots (26). But in 1966, fairly large amounts of extractable  $\text{NH}_4\text{-N}$  were still present in humus layers where urea was added (Table 2). These results indicate that the urea was converted in less than two years to  $\text{NH}_4\text{-N}$ , that pH increased, and that the  $\text{NH}_4\text{-N}$  was not absorbed by the microbes but the greatest part remained adsorbed in the humus horizon, in a form available to the trees, for up to five years.

In 1964, the potential for ureolysis was higher in the humus layers of the 450 kg urea-N/ha fertilized plots than in the humus layers of the unfertilized plots (Table 2). Ureolysis rates, abundance of ureolytic microorganisms, and the number of microorganisms with a high urease-producing activity were greater in the humus layers from the treated plots than in those from the untreated plots.

In 1964, the potential for total biological activity was also higher in the humus layers of the 450 kg urea-N/ha fertilized plots than in the unfertilized plots. This was indicated by the difference in the total number of bacteria and fungi (Table 2). Marked reduction in these numbers occurred at a higher dose of  $^{60}\text{Co}$  radiation in the urea-treated layers than in the untreated humus layers (27), also indicating a possible qualitative microbial change following urea fertilization. A possible qualitative microbial change was also expected from the analysis of the number of ureolytic microorganisms (28) and of their capacity to produce urease (25). A qualitative fungal change was observed (22). For example, *Penicillium* spp. increased their representation by about 30 percent in the L layer following urea fertilization.

#### Effects of Thinning in the Unfertilized Plots of the Stand

In 25 percent and 50 percent thinned and unfertilized plots of the stand, growth and foliar N concentrations of black spruce trees, litter-fall amounts, light intensity and rainfall interception, and growth and

Table 2. SUMMARY OF THE CHEMICAL AND MICROBIOLOGICAL ANALYSES CARRIED OUT ON THE HUMUS HORIZONS

Humus layer	Total N (%)	C/N	pH	Extractable	Ureolysis rate <sup>1</sup> (ppm NH <sub>4</sub> -N)	Total number (million/g)	
				NH <sub>4</sub> -N (ppm)		Bacteria	Fungi
<i>Untreated plot</i>							
L	0.85	59	4.8	30	1,400	228	10.2
F <sub>1</sub>	0.92	55	3.5	30	850	50.0	6.8
F <sub>2</sub>	0.85	59	3.3	30	700	46.1	5.8
H	1.70	30	3.1	30	.....	.....	.....
<i>Plot treated with 450 kg urea-N/ha in 1961</i>							
L	(1966) 1.33	(1966) 38	(1966) 4.9	(1966) 30	(1964) 1,800	(1964) 250	(1964) 45.3
F <sub>1</sub>	1.33	38	4.0	210	1,200	167	24.6
F <sub>2</sub>	1.15	44	3.6	200	1,000	149	10.6
H	1.73	29	3.3	300	.....	.....	.....
<i>Samples (from untreated plot) incubated for 3 days with 3,500 ppm urea-N in the laboratory</i>							
L	.....	.....	6.7	2,440	.....	1,470	12.1
F <sub>1</sub>	.....	.....	5.1	2,630	.....	168	7.6
F <sub>2</sub>	.....	.....	4.7	2,860	.....	64.7	5.9

<sup>1</sup> NH<sub>4</sub>-N (ppm) liberated in 8 hours at 20°C after addition of 3,500 ppm urea-N.

Table 2. SUMMARY OF THE CHEMICAL AND MICROBIOLOGICAL ANALYSES CARRIED OUT ON THE HUMUS HORIZONS (Continued)

Humus layer	Number of ureolytic		Number having high urease producing activity		Number (million/g)	
	Bacteria (million/g)	Fungi (million/g)	Bacteria (million/g)	Fungi (million/g)	<i>Penicillium</i> spp	Yeasts
<i>Untreated plot</i>						
L	86.6	9.2	13.7	2.8	2.0	2.2
F <sub>1</sub>	22.5	5.3	2.5	1.6	3.5	1.7
F <sub>2</sub>	9.2	4.6	2.2	1.4	3.8	0.6
<i>Plot treated with 450 kg urea-N/ha in 1961</i>						
L	(1964) 130	(1964) 44.4	(1964) 28.8	(1964) 35.4	(1964) 20.4	(1964) 12.7
F <sub>1</sub>	103	23.1	30.2	19.1	12.3	3.4
F <sub>2</sub>	73.5	9.8	30.5	6.3	6.7	1.5
<i>Samples (from untreated plot) incubated for 3 days with 3,500 ppm urea-N in the laboratory</i>						
L	788	11.3	146	4.2	.....	.....
F <sub>1</sub>	89.8	6.7	12.9	2.6	.....	.....
F <sub>2</sub>	27.4	4.7	5.1	2.0	.....	.....

nutrient content of mosses were determined. Moreover, in these thinned plots and in the clear-cut area, humus temperatures were compared.

Black spruce trees showed a small but significant growth response to thinning (Table 3). Also, in 1965, trees from plots thinned in 1961 showed increased foliar N levels (Table 3 and Figure 6). Early small increases in growth and in foliar N levels may be expected following thinning in a stand which showed a growth response to N fertilization. The addition of logging slash with a high C/N ratio might be expected to decrease the rate of mineral N production temporarily (1, 10). Growth response and increased foliar N levels may have been due to utilization by fewer trees of the limited amount of N which was available from the humus.

Table 3. EFFECTS OF THINNING ON SOME TREE AND ECOLOGICAL PROPERTIES

Thinning intensity (%)	Diameter growth 1961-66 (mm)	Foliar N concentration		Light intensity interception in 1965 (%)	Rainfall interception in 1965 (%)
		in current year needles	in 1965 (% dry-wt. basis)		
0	4.39	0.85	1,280	90	40
25	4.53	0.92	780	80	30
50	4.50	1.20	410	60	20

### Regeneration

After four years, there was no black spruce regeneration in the unthinned, 25 percent thinned, and 50 percent thinned plots (45). In the clear-cut area, a few black spruce, pin cherry (*Prunus pensylvanica* L. f.), and trembling aspen (*Populus tremuloides* Michx.) seedlings were found, mainly in rotten wood, shaded spots, or on exposed mineral soil.

### Mosses

In 1964, the weight of green moss increased with greater thinning intensity up to 50 percent (Table 4). The N content of the mosses decreased from 49 kg/ha in unthinned plots to 34 kg/ha in thinned plots. The initial source of N for mosses is considered to be from N contained in dust, precipitation, and foliage leachates (33). In the unthinned plots, a rough estimate of the N addition in precipitation was 5.3 kg/ha (45). An additional 2.0 kg N/ha were washed from the tree crowns. In the same plots, the annual N requirements of the moss

Table 4. EFFECTS OF THINNING ON SOME MOSS PROPERTIES DETERMINED IN 1964

Thinning intensity (%)	Number of moss segments (million/ha)	Weight of green mosses (kg/ha)	Weight of dead mosses (kg/ha)	Nutrient content of green and dead mosses (kg/ha)				
				N	P	K	Mg	Ca
0	93	1,000	4,200	49	10	32	3	23
25	110	1,100	4,000	40	10	29	3	20
50	100	1,300	3,500	34	7	27	2	18

layer were estimated at 5.0 kg/ha, assuming green moss segments constitute two years' growth.

#### *Humus horizon*

During the 1962 growing season, the mean humus temperature 2 cm below the surface was 9, 11, and 14°C in the unthinned, 50 percent thinned, and clear-cut plots, respectively (34). Following clear-cutting, humus temperature at this depth can reach 27°C. On the surface of the humus horizon, 49°C was recorded on certain days. In spite of warmer conditions after thinning and clear-cutting, slash decomposition was slow. In 1966, in the thinned plots much of the slash was found buried by feather mosses. The increased rate of slash decomposition which could have resulted from increased humus temperature was greatly reduced by exposure of the humus to drying conditions after cutting the overstory.

The thermal insulating capacity of the humus horizon was found to vary seasonally with respect to both temperature lag and the freeze and thaw relationship of the humus horizon. The uncut stand was 25 percent cooler and 45 percent warmer than the clear-cut area in the summer and winter, respectively. The heavy thinning increased the mean summer temperature at all depths in the humus horizon and might be expected to prolong the growing season of the roots.

#### Combined Effects of Thinning and Urea Fertilization

Growth and foliar N concentrations of black spruce trees were measured in 25 percent and 50 percent thinned plots of the stand fertilized with 110 or 450 kg urea-N/ha.

#### *Stand*

At the end of five growing seasons, the heavily thinned, heavily fertilized plots showed double the rate of the tree growth of the untreated control plots (Table 5). In 1965, foliar N concentrations were also much higher in the thinned and fertilized plots than in the unferti-

lized plots (Figure 6). This response indicated that trees were still short of available N following thinning.

Table 5. PERCENTAGE VOLUME GROWTH DURING FIVE YEARS FOLLOWING TREATMENT

Urea-N added in 1961 (kg/ha)	Percent thinning in 1961			
	0	25	50	Weighted mean
0	6.5	5.6	12.0	7.2
110	9.4	12.5	14.1	11.4
450	9.6	13.6	19.0	12.9
Weighted mean	8.5	10.4	14.9	

### *Regeneration*

In 1963, large numbers of black spruce germinants appeared in patches where urea had killed the feather mosses (42), but nearly all seedlings died the following year, even on areas 50 percent thinned.

### **Planting in the Clear-cut Area**

Following clear-cutting in 1961, groups of wilding black spruce seedlings, four to six years of age, were planted to assess the availability of N from the exposed humus. Planting treatments were designed to test the effects of mixing the humus and mineral soil, of adding 450 kg urea-N/ha plus 70 g superphosphate placed in bands around each tree, and of combinations of these two treatments. At the end of the second growing season, survival rates were less than 50 percent for all treatments except the soil and humus mixed, which was 68 percent (44).

Analyses of variance of 1965 leader lengths showed that those of trees in the control blocks were significantly shorter (Table 6). Leader lengths of trees in the other blocks were not significantly different at the 5 percent level.

By the fall of 1966, all surviving trees were vigorous and healthy, with blue-green needles. Foliar analyses indicated very high N levels in current needles from trees in all treatments, even those that had received no N (Table 6). Thus, planted trees were not deficient in N, although 65-year-old spruce trees in the stand a few meters away were deficient. These planted trees were growing mainly in the humus horizon which, even after five years, showed little decomposition of needles and twigs, although feather mosses had died and partially decomposed.

Table 6. LEADER GROWTH (BY TYPE OF FERTILIZER TREATMENT), FOLIAR NUTRIENT CONCENTRATIONS, AND PROPORTIONS IN PLANTED BLACK SPRUCE

Treatment	Leader length in 1965 (cm)	Nutrient concentrations and proportions* in 1966				
		N	P	K	Mg	Ca
Control	16.8	Range in the four treatments	1.74-2.01	0.29-0.39	Concentrations	0.10-0.11
Mix humus plus soil	20.7			0.46-0.49	0.37-0.45	
Urea plus P	20.4			Proportions		
Mix plus urea plus P	21.1		100	14-21	23-38	5-6
* Optimum proportions (15)		100	8-15	5-100	5-10	5-10

Phosphorus levels were also high, even in trees which had received no P. However, K levels were low in comparison with values or proportions (15) for better nutrition. Probably most of the N available to the black spruce seedlings originated from humus decomposition and precipitation. The low survival rate was possibly due to moisture deficiency.

#### Black Spruce Seedling Response in Greenhouse Studies

To further determine how the nutrient reserves in the humus layer could be mobilized, the growth of black spruce seedlings planted in intact cores of the humus horizon was measured. Treatments involved N additions as urea, simulated burning by ashing one third or two thirds of the humus and adding the ash to the remaining humus, and fertilization with powdered chemicals or nutrient solutions. After nine months' growth, a small but not significant response in growth to either N additions or ash elements was found. However, the combination of N and ash, or the approximate major elements of the ash, produced significantly greater growth than any of the treatments involving additions of either N or ash alone.

For these seedlings, almost entirely rooted in the humus horizon, when the amount of added N only was increased from 110 to 450 kg/ha, the proportional levels of the ash elements, particularly K, were diluted (Table 7.) Further additions of humus ash or powdered chemical fertilizer increased the proportions and particularly raised the proportional level of K to that suggested as optimum (15).

These results indicate that in spite of a high C/N ratio in the humus horizon, trees planted in this horizon have an adequate supply

Table 7. FOLIAR N CONCENTRATIONS AND NUTRIENT PROPORTIONS OF SEEDLINGS GROWN IN CORES IN THE GREENHOUSE AFTER TREATMENT WITH UREA-N

Treatment (kg/ha)	N Concen- tration (% dry wt basis)	Proportions*				
		N	P	K	Mg	Ca
110-N .....	1.03	100	12	29	7	28
450-N .....	1.79	100	5	14	3	10
110-N plus ash from one third of the humus horizon .....	0.75	100	26	48	11	41
110-N plus 330-K, 320-Mg and 420-Ca .....	1.59	100	7	57	14	27
*Optimum proportions suggested (15) .....	.....	100	8-15	50-100	5-10	5-10

of mineral N. This humus contains a large reserve of N, a large part of which is in feather mosses. These feather mosses mineralize N readily upon decomposition (1). Planted trees must have chiefly used this mineral N.

#### Nitrogen Metabolism in the Humus Layers

To study the N metabolism of the urea added in the field in 1961, humus samples from the unfertilized plots of the stand were incubated in the laboratory with 3,500 ppm of urea-N. On a field basis, this is equivalent to 450 kg urea-N/ha.

When no urea was added in the laboratory, there was some accumulation of  $\text{NH}_4\text{-N}$  in the L layer (24) and in the H layer. It has been shown that feather mosses when added to humified material mineralize N rapidly during incubation (1). These results indicate that the mosses also mineralize N when incubated alone. There was little change in all other chemical and microbiological properties determined during laboratory incubation of humus without urea addition (26, 28, 30).

Because urea is very soluble, it could have easily diffused throughout the soil solution when added to moist humus. A small part could have been adsorbed by the humus (2, 4). Added urea was rapidly converted to  $\text{NH}_4\text{-N}$  (Table 2) and hydrolysis was complete after three days (26).

When urea was added in the laboratory, much  $\text{NH}_4\text{-N}$  was present in the humus layers after a three-day incubation period (Table 2). Since the pH remained below 7.0 even in the L layer, it is unlikely that the mineral N remained long in the  $\text{NH}_3$  form, that  $\text{NH}_3$  was volatilized, or that  $\text{NH}_3$  was irreversibly fixed on organic debris.

The determinations of  $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$  showed that nitrification was not evident during a 42-day incubation period after urea addition (26). This absence of nitrification appears desirable considering the readily leachable nature of the  $\text{NO}_3\text{-N}$ . Immobilization of the  $\text{NH}_4\text{-N}$  released on hydrolysis of the urea decreased with depth (Table 2). This was to be expected, considering the varying degrees of decomposition of the layers and the greater availability of readily decomposable organic materials in the L layer. Microbial demand for N appeared easily satisfied by the level of N applied, and much remained in a form that was exchangeable and presumably available for microbial nutrition.

The addition of urea appreciably increased numbers of bacteria and fungi (Table 2). The different individual increases observed probably reflected differences in N availability and in initial pH values, the differential pH increases following urea addition, and the amounts of readily available organic matter. Layers showing the largest popula-

tion increase following urea addition exhibited the most immobilization of the liberated  $\text{NH}_4\text{-N}$ .

Appreciable changes in total numbers did not occur from the 3rd to the 42nd day of incubation following addition of urea in the laboratory, although the N immobilization continued (28). After three days, it might be considered that a dynamic equilibrium was maintained between the death and proliferation of cells, an equilibrium consistent with the significant biological N interchange reported in the same humus material (5).

Increases in numbers of ureolytic bacteria and fungi, which occurred after urea addition, were roughly parallel to the increases in total numbers (Table 2). However, as a percentage of the total, the ureolytic bacteria and fungi increased by about 5 percent during the early part of the incubation period.

Comparisons are often made between soil-enzyme measurements on the one hand, and soil type, fertilization, microbial counts, and respiration on the other. The results in this paper show a direct relationship between rate of ureolysis and degree of decomposition of the different layers of the humus horizon, as well as between the numbers of total and ureolytic microorganisms (Table 2).

### Discussion and Conclusions

A large portion of the nutrient capital in the forest ecosystem is often contained in the humus horizon, and the rates at which nutrients are mineralized, adsorbed on colloids, and absorbed by trees are of prime significance to forest productivity.

The purpose of this study has been to examine the means of increasing tree and regeneration growth and avoiding, or at least decreasing, the accumulation of organic matter on the mineral soil in an upland black spruce stand with a thick humus accumulation. In this stand, rate of tree growth and ease of establishment of vigorous black spruce regeneration is very closely related to the condition of the humus horizon. An improved rate of tree growth could be expected by thinning, by N fertilization, or by both. Thinning could result in a greater release of mineral N by increasing, or changing the character of, the microbial activity through an increase in temperature and a decrease in moisture. Nitrogen fertilization, in a mineral form or in an organic form readily transformed into a mineral form, could also improve tree growth since black spruce trees were showing N deficiency. Low foliar N levels were noted in this stand in which the living trees contained 258 kg N/ha, the living mosses 9.9 kg N/ha, and the humus horizon 890 kg N/ha.

Improved regeneration establishment and growth could also be expected by thinning and N fertilization. Both of these treatments might be expected to result in an increased rate of humus decomposition and in a smaller accumulation of humus on the mineral soil. The difficulties of establishing valuable black spruce regeneration, either naturally or artificially, on large accumulations of humus have long been recognized in reproduction works and surveys in eastern Canada (3, 14). Current numerous Canadian trials and experiments with scarification, seeding, and planting show these difficulties (16). Swedish experience (41) with Norway spruce (*Picea abies* (L.) Karst.) and Canadian experience with black spruce have indicated the following conclusions on regenerating these species: a) scarification of soil and seeding or planting is worth trying, but specialized equipment is needed for mechanically and biologically efficient scarification work; b) vigorous stands can sometimes be established by burning and seeding or planting; c) natural advance growth is often not worth keeping and generally gives rise to only patchy, slow-growing stands of layer origin; d) clear-cutting is an accepted regeneration, silvicultural system; e) on clear-cut areas, spruce seedlings germinate and survive only on shaded and rotten wood microsites; and f) clear-cut areas not promptly restocked may become covered by ericaceous heathland vegetation. These findings are not especially related to the characteristics of the species, but they are largely a result of the type of humus formation, soils, and soil processes.

A detailed review of literature showed many studies of fertilization with urea (23) and also pointed out possible advantages of urea over all other N fertilizers in improvement of tree growth (7, 8, 9, 11, 12) on acid forest humus (13, 17, 18, 35).

In the five years following fertilization of the stand with 110 and 450 kg urea-N/ha, tree diameter growth increased in rate following increases in the foliar N concentrations. The microbial population also increased. Apparently, two factors contributed to the tree and microbe responses: first, the occurrence of available N, and second, the increase in pH brought about by the mineralization of urea.

It is interesting to speculate why a stand of trees growing on such a large accumulation of organic N is deficient in N. Some insight can be obtained from the following estimates of the cycling of N in the untreated stand:

	kg/ha
Net mean annual uptake by trees between 1896 and 1961 .....	18.5
Net uptake by mosses in 1964 .....	5.0
Return in litterfall in 1963 .....	6.6
Supply in rainfall in 1965 .....	7.3

	<i>kg/ha</i>
Net accumulation found in trees in 1961 .....	258
Net accumulation found in mosses in 1964 .....	10
Net accumulation found in humus in 1961 .....	890

Thus there was a large quantity of N in the humus to meet the relatively modest, 18.5 kg/ha, mean annual requirements of the trees. Most of the N in the humus was in an organic form since the amounts of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$  were negligible in the four humus layers. As long as N is in an organic form, it is unavailable for plants except possibly through mycorrhizal action. Mycorrhiza were observed in the humus. Nearly half of the annual N supply in the rainfall was  $\text{NO}_3\text{-N}$ , consequently available. The rest of the annual N return in rainfall and the annual N return in litterfall, which is in organic form, is probably unavailable. The balance of the annual uptake by the trees thus probably came from the mineralization of the organic N in the humus. The absence of  $\text{NH}_4\text{-N}$  at sampling time indicated that it was absorbed by plants as soon as it occurred in the humus.

An incubation experiment in the laboratory indicated a release of  $\text{NH}_4\text{-N}$  in the litter and in the humified layer. Accumulation of  $\text{NO}_3\text{-N}$  and  $\text{NO}_2\text{-N}$  was not observed. Among organic debris, mosses were shown to have the greatest ability to supply available N (1). Even though mosses had a C/N ratio ranging between 35 and 59, they started to liberate mineral N as soon as they were mixed with humified materials. But black spruce needles delayed production of mineral N for 12 weeks.

Detailed study of the humus horizon indicated a very dense network of live spruce roots in the humus. This upward growth of tree roots into the L layer is probably due to a greater availability of nutrients at the base of the feather moss layer.

Some evidence indicates that the primary source of the 1,158 kg N/ha accumulated on this site was nonsymbiotic N fixation. No evidence of N accumulation in the humus in the field was obtained between 1961 and 1966. Studies on the incorporation of  $^{15}\text{N}_2$  by isolated humus samples are in progress.

Nitrogen fertilizers such as urea are likely to inhibit fixation of N gas by microorganisms which have the ability to utilize  $\text{NH}_3\text{-N}$  or  $\text{NH}_4\text{-N}$  and sometimes  $\text{NO}_3\text{-N}$  (6). The duration of this inhibition depends on the speed of disappearance of the mineral N. As there was still some mineral N in the humus in 1966, nonsymbiotic N fixation did not likely occur and the larger amount of N in the humus of the 450 kg N/ha fertilized plots was only from the added N.

Losses of N were probably small. Loss due to leaching was approximately 1 kg/ha in 1963. Loss due to leaching, as well as loss due

to volatilization, was expected to be small between 1961 and 1966 since the amounts of  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ , and  $\text{NO}_2\text{-N}$  were negligible in the four humus layers.

Results from laboratory incubation experiments showed that urea was rapidly hydrolyzed, liberating  $\text{NH}_4\text{-N}$  which raised the pH of the humus. However, the rise in pH was not sufficient to create conditions where  $\text{NH}_3$  volatilization could be expected to be important, and neither was there any evidence of nitrification. Thus, there was little possibility of N loss through volatilization or leaching. The  $\text{NH}_4\text{-N}$  remained in exchangeable form in the humus and significant quantities were present in the field up to five years after fertilization. This  $\text{NH}_4\text{-N}$  was presumably available for microorganisms and for plants if required.

Since the C/N ratio of the humus was about 50,  $\text{NH}_4\text{-N}$  would be expected to be rapidly utilized and hence immobilized (1, 10). The reasons this did not occur to a great extent could be due to the low pH environment, the presence of inhibitory substances, or the low susceptibility of the humus constituents to enzymic breakdown. The small amounts of immobilization that did occur decreased with depth and were perhaps related to the decrease in availability of suitable carbonaceous materials.

Microbial populations were roughly proportional to the initial rates of urea hydrolysis and also decreased with depth. The population increased during the period of active ureolysis, but thereafter it remained more or less constant in agreement with the low rate of immobilization.

In the five years following thinning at both intensities, the rate of diameter growth increased and there were increases in the N concentrations in the needles. Thinning can be considered as making more of the limiting factor, i.e., N, available to the remaining trees. Since thinning increased the mean humus temperatures only  $2^\circ\text{C}$ , it is not considered likely that this increase would appreciably increase the nitrogen availability in the humus during a five-year period. In the long run, decomposition of slash from thinned trees on the plots is also a form of N fertilization.

Economically, thinning before fertilization means that growth response due to fertilization will be concentrated on stems which will probably all be merchantable. In unthinned stands, growth increases due to N fertilization result in increased growth of small and suppressed trees, which may eventually die before harvest, as well as the larger trees. This factor should be considered in calculations of economic feasibility of fertilization, particularly when planning to ferti-

lize more than 10 years before cutting very dense stands of black spruce with more than 5,000 stems/ha.

### Summary

Plots of a 65-year-old black spruce stand were thinned (two intensities—25 or 50%) and/or fertilized (two levels—110 or 450 kg N/ha). An additional area was clear-cut and planted.

The trees responded to both thinning and fertilization. Increases of up to 100 percent in diameter growth following fertilization and thinning were found at the end of five growing seasons. These were associated with increased levels of foliar N. Seedlings planted in the clear-cut area and in intact cores of the soil in the greenhouse did not respond to fertilization. Thinning resulted in faster growth of feather mosses on the forest floor. Clear-cutting resulted in increased humus temperatures, a lengthened growing season, and an increased rate of thawing of the soil.

The humus microbial population increased in number and activity, and changed in composition, following urea fertilization. The added urea was rapidly converted to NH<sub>4</sub>-N by the action of urease in both natural and irradiated humus samples. Urease activity was studied in the humus.

The operation of the N cycle and the availability of N from the raw humus layer are discussed in relation to the growth and foliar nutrient contents of black spruce trees and seedlings following N fertilization.

Urea N fertilization, either with or without thinning, is recommended for stimulating growth of black spruce trees on upland raw humus sites.

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